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USE OF THE CHICK EMBRYO IN THE STUDY OF CRYPTOCOCCOID INFECTION*

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Introduction

Considerable work has been done with the chick embryo commercially after ingenious studies made by research workers. Vaccines for several virus diseases have been made by the use of the chick embryo, such as, smallpox (1), equine encephalomyelitis (2), and also for the Rickettsial diseases of Rocky Mountain spotted fever and typhus (3).

This is the first time we have had an interesting bacteriological problem in our laboratory which has given us the opportunity to use the chick embryo since our first work with it in 1940 (4).

Although the chick embryo is a laboratory animal cheap, uniform, self-nourishing, protected from incidental diseases, to date there have been few reported studies made employing this medium in current clinical bacteriology along with the commonly used cultural methods. Blattner, Keys, and Hartman (5) have used the chorio-allantoic membrane for primary isolation of organisms from spinal fluid, and during chemotherapy (sulfonomides) egg culture

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Prize Award (first prize).

showed positive cultures when blood agar cultures were sterile. In their study when organisms were not seen in direct smears, the growth on blood agar was much delayed or scanty, but on the chorio-allantoic membrane of the chick embryo the growth occurred within 24 hours.

In our first use of the chick embryo we were chiefly setting up the equipment and learning the technic, but this time we are using it in addition to the regular procedures for identifying fungus-like organisms.

With past experience in the use of the chick embryo and our present work with it, we are satisfied that we technicians can satisfactorily use it in the same manner as inert media and laboratory animals.

Moore (6) in 1939 used the chorio-allantoic membrane of the developing chick in the study of pathogenic fungi and found some degree of specificity as found in infection in man.

Source of Organism

The organisms were first observed in the sputum of a mental patient who developed pneumonia 9 months after institutionalization. Clinically the patient's condition seemed to be the usual bronchopneumonia, but in the sputum there were several kinds of bacteria both gram-positive and gram-negative (probably mouth bacteria) with an occasional large gram-positive cell-like organism (Fig. 1). The patient's course was rapidly downhill, and he died before a sputum specimen was obtained for culture. At autopsy, the purulent exudate from the bronchus was collected under sterile conditions by the pathologist. Direct smears of this exudate (Fig. 2) showed the same cell-like organisms as were previously found in the sputum but in large numbers. Cultures were made and the organism isolated.

Isolation of Organism

Cultures of the autopsy material were made in dextrose broth enriched with brain and on a blood agar plate. The broth became cloudy with some sediment within 24 hours and showed a variety of bacteria and a few of the gram-positive cell-like structures in question. The blood agar plate grew heavily within 24 hours with

opaque colonies surrounded by a translucent zone and a few hemolytic colonies. The hemolytic colonies showed a pure culture of gram-positive cocci in clusters (staphylococci). The other colony showed a mixture of gram-positive cocci and the organism in question.

Five days later, the colonies from the blood agar plate were inoculated onto a maltose Sabouraud plate and a maltose Sabouraud slant; this media being chosen because the gram-positive cell-like structures appeared to us to be a fungus and we wished both to encourage its growth and to discourage the growth of the staphylococci with which it seemed to be inextricably mixed. In 24 hours there was no growth, after 48 hours there was slight growth. On the 3rd day a gram stain was made showing a few forms cell-like in structure having many coccoid forms, and some gram-positive cocci in clusters.

At the end of 16 days a subculture was made by streaking from the maltose Sabouraud slant a maltose Sabouraud plate enriched with blood. Within 24 hours two types of colonies appeared, one of which proved to be a typical staphylococcus albus and the other a pure culture of the cell-like structures as originally observed in the sputum.

Characteristics of the Organism

After 72 hours on the original blood agar plates the majority of the organisms had from 2 to approximately 8 coccoid bodies, smaller in size but larger in number than in the 24 hour culture. Grossly the colonies were essentially the same except for absorbing some of the blood pigment, appearing muddy brown in color. Also the entire blood plate turned muddy brown color.

Then they were transferred to a Sabouraud plate and slant with special attention for hyphae, mycelium, spores, etc. In five days colonies on maltose Sabouraud plate appeared glossy, white with a translucent zone, dew drop, smooth and round. After 16 days there was no evidence of mycelium. The gram stain showed no appreciable change except in variation of staining.

At this time the colonies were subcultured on the maltose Sabouraud, enriched with blood, plate to see whether or not the organisms would return to their original form. Within 24 hours colonies

appeared. They were glossy, translucent, dew drop, round, elevated, moist, smooth-surface, slimy colonies which in gram stain were large cell-like structures with prevalence of fine connecting threads (Fig. 3). In 72 hours these organisms had changed from the large, heavy, gram-positive, uninuclear form to the other form having 4 to 20 coccoid bodies within a cell-like structure (Fig. 4). The fine connecting thread had disappeared.

The pure culture of organisms obtained from the blood Sabouraud plate were cultured consecutively in three dextrose broth media enriched with brain, two blood agar, four Sabouraud, nine blood Sabouraud media for its growth and change from one form to the other. Through several subinoculations it was found that the organisms grew within 7 hours if they were uninuclear form and if the media were enriched with blood or brain, and after 7 hours they began changing into the multicoccoid form. On blood Sabouraud the organisms changed within 4 hours, after abundant growth at 18 hours, from the uninuclear form to the multicoccoid form. In broth this change occurs much slower, in some cases as long as 4 days. At no time have I seen the organism change from the multicoccoid form to the uninuclear form without a transfer from one medium to another. If the organisms were multicoccoid forms, they required from 18 to 24 hours for growth. In some instances on the Sabouraud without blood they required 48 hours or longer to produce growth.

In summary, the general appearance of the organisms is that of a cell-like structure varying in size from a coccus to a lymphocyte staining from heavily gram-positive to a light purple. The nuclear structure may stain very heavily gram-positive appearing as a single or diplo structure, but older cultures show a nuclear structure of 2 to 20 coccoid bodies within a gram-negative staining cytoplasm-like structure. The heavier staining organisms have a fine gram-negative thread connecting one organism to another. This thread seems to disappear on older cultures.

Besides the gram stain, which was used for the entire study, Best's carmine and silver stain and spore stain by Maneval (Dodge) were attempted. These stains showed no structures not seen in the gram stain.

Hanging drop cultures were also made from broth, blood Sabouraud and Sabouraud media. From blood Sabouraud and Sabouraud

the organisms appeared as small refractive bodies in pairs or in fours. In broth they were essentially the same except for some very interesting bodies which were discovered to be artifacts two days later.

A guinea pig was inoculated with 0.5 cc of brain broth culture intraperitioneally. This broth culture was 12 days old, a subculture from the blood Sabouraud from which the organism was first isolated. This culture was used because the form of the organism was furthest removed from the original form as found in the sputum. The guinea pig is well at time of writing.

Fermentation tests with sucrose, maltose, lactose, mannose, fructose and glucose were made. No gas was formed in any of the media, but there was acid formation in all. In sucrose, maltose, and lactose, the sediment was heavier, a mucinous sediment lying just above a powdery sediment; while in mannose, glucose and fructose there was the powdery sediment only. The powdery sediment in gram-stain showed the multicoccoid form (see Fig. 4) while the mucinous layer showed the uninuclear form with some fine connecting threads (see Fig. 3).

Comparative cultures were made on several media with the following results:

On potato slant the colonies were shiny, white, moist, slightly mucoid and smooth. There was abundant growth within 22 hours with no appreciable change thereafter. Gram stain showed the multicoccoid form even as early as 22 hours.

On Difco wort agar there is no growth yet after 19 days.

On nutrient gelatin the colonies are white, moist, somewhat slimy. Along the line of stab there are small round white colonies. No liquefaction yet (21 days). The majority of the organisms are the multicoccoid form.

On the conservative media, which is media of poor nutritive value to bring out mycelium or spores, there is no growth yet (26 days). Potato infusion, carrot infusion with calcium sulfate, carrot slant, and corn meal agar media were used.

The alcohol consumption test and the behavior of the organism on litmus milk is under investigation but the results are not definite as yet.

Experimental Procedure

Our equipment and chick embryo technic have been described previously in detail (4). Briefly, fertile eggs are incubated for 12 days; on the 12th day a small window (Fig. 5) is cut out of the shell of the embryo-containing egg. Beneath the shell and exposed in the window is the chorio-allantoic membrane (seen in Fig. 6) which membrane is used bacteriologically as if it were the surface of a solid media culture plate. The surface of the membrane is inoculated through the window, the window is rimmed with vaseline-paraffin to seal the sterile cover-slip which is placed over the window. This is known as the Goodpasture-Buddingh technic.

For the further investigation of the aforedescribed organism three chick embryo experiments were set up.

The first and second experiments were for the purpose of studying the two different forms of the organism on the chorio-allantoic membrane. For these, nine 12-day embryos constituting Series I were inoculated with a platinum loopful of a 24-hour brain broth culture which had the uninuclear form of organism. A second group of nine 12-day embryos constituting Series II was inoculated with a platinum loopful of a 23-day old brain broth culture which had the multicoccoid form of organism.

After inoculation, the eggs were put back into the chick incubator for observation. Every 24 hours, smears from the chorio-allantoic membranes were made noting especially the form of the organisms most prevalent in each case. These observations were recorded on the charts. Also, one embryo from the series inoculated with the 23-day culture and one embryo from series inoculated with the 24-hour culture were sacrificed: from the embryo's heart, blood cultures were made in broth, and the embryo and membrane were placed in 10% acetic Zenkers for pathological studies.

To culture the heart's blood, the heart is exposed by a long midline incision of the embryo, which had been removed from the shell and placed in a sterile Petri's dish. The incision is made with small sterile scissors. The heart is pulled up into the incision with sterile forceps and the heart's surface is sterilized by searing it with a red-hot knife blade (Fig. 7). This seared area is pierced through with a capillary pipette (Fig. 8) which enters the heart chamber and draws up a small amount of blood. This blood is then cultured

in selected media, which in our experiment was brain broth.

At the end of 48 hours the series of eggs inoculated from the different broth cultures, that is, Series I, from the 24-hours culture having the uninuclear form, and Series II, from the 23-day culture having the multicoccoid form, showed no appreciable difference as having had a different source of inoculum. Therefore, only one from either of Series I or Series II of inoculated embryos were sacrificed at 48 hours, 72 hours and 4 days; and the two experiments are combined on Chart I, which records the individual findings on Series I and II.

For the third experiment, started at the same time as those just described, four 12-day old embryos were inoculated with the 24-hour broth culture. This series, A-J, was used to study the effect upon the organism and the embryo of continuous transfers from one generation of eggs to another generation every 24 hours. Each generation of eggs consisted of from two to four 12- to 14-day embryos and the study was through 9 serial transfers, thus using nine groups, or generations, of embryos.

Smears at the end of 24 hours were made from the four eggs and by gram-stain that egg showing the most interesting picture was used to inoculate the second generation, generation B. Then at the end of another 24 hours smears were made from the B eggs and by gram-stain an egg of the B series selected for a source of inoculum of the C eggs, etc. (see Chart II). After being used for serial subculture the eggs used in the 9 generations were either sacrificed or had died and the embryos were treated in the same manner as in Series I and II.

From this A-J series, one embryo was sacrificed at 5 day, 6 day and 7 day subinoculation to complete the Series I and II in which the eggs had not survived. Material from the chorio-allantoic membrane of one egg from the 9th generation, 48 hours after inoculation, was inoculated into broth and sugar media for fermentation tests. Smears from chorio-allantoic membranes were made daily from the eggs and the findings put on work charts (see Chart II).

The dead embryos in the entire set-up were taken care of in the same manner as the sacrificed embryos. Brain-broth was the medium used throughout the chick embryo study, because of the organisms

immediate growth in it, and the organisms longer survival period in it.

Experimental Results

In the transference through 9 generations of embryos, the organisms when recultured showed their original characteristics in broth culture, on fermentation tests with six sugars, and on gram-stain.

The gross colonies, or lesions, on the chorio-allantoic membrane varied greatly in the 45 inoculated embryos. Several membranes showed suspicious growth as a small yellowish-white crusted area (Fig. 6) and one dead embryo showed a typical colony such as was described on blood Sabouraud plate. The gross lesions occurred in not less than 4 days, in one embryo on the 5th day, and in one on the 6th day. Five of the inoculated embryos had dry membranes and very few organisms.

Smears made from the chorio-allantoic membranes showed pictures which were inconstant (Fig. 9). There was no constant form of organisms persisting in the length of time of infection by either inoculum. No contamination occurred in this study. The organism changed from uninuclear form to the multicoccoid form with some of the multicoccoid form being intracellular. In the longer period of infection, the majority of the organisms were the multicoccoid form. The multicoccoid form of inoculum became uninuclear form in from 24 hours to 72 hours. The uninuclear form of organism became multicoccoid within 24 hours. However, in most instances both forms were present in the chorio-allantoic membrane after 24 hours regardless of the form of inoculum.

Three heart's blood culture from 11 sacrificed embryos were positive. Nine heart's blood cultures from 19 dead embryos were positive. In the dead embryos, where the blood did not freely flow up the capillary pipette, though precautions were taken in sterile technic, there is a question of possible contamination from amniotic fluid.

The survival period of the embryos after inoculation of the chorio-allantoic membrane varied, the 7th day sacrifice being the longest life of the embryo.

The virulence of the organism was inconstant throughout the study and any changes in virulence by successive transfers were not

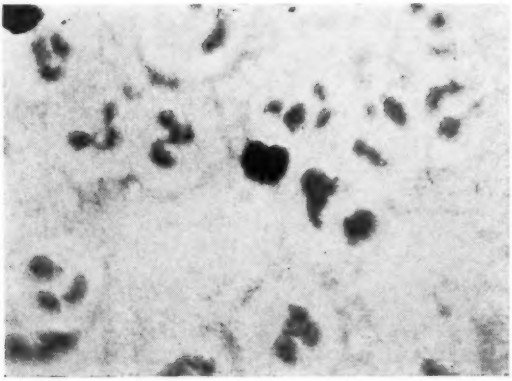

Fig. 1
Sputum showing an occasional large gram-positive cell-like organism.

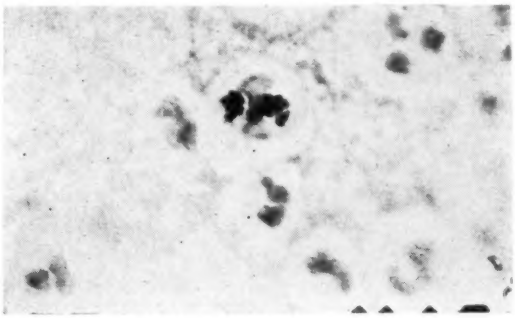

Fig. 1
Sputum showing intracellular gram-positive cell-like organism.

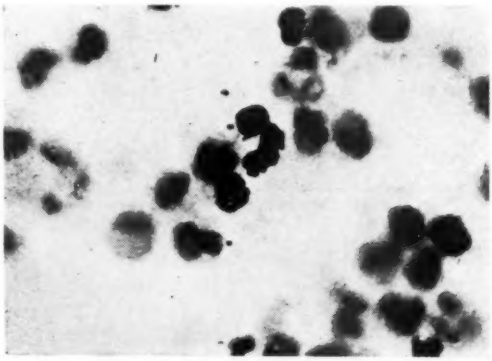

Fig. 2
Purulent exudate removed from bronchus at autopsy.

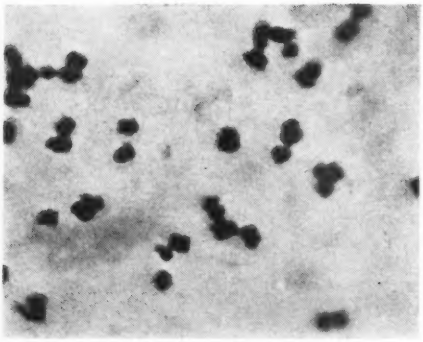

Fig. 3
Uninuclear form of organisms, some having fine connecting thread.

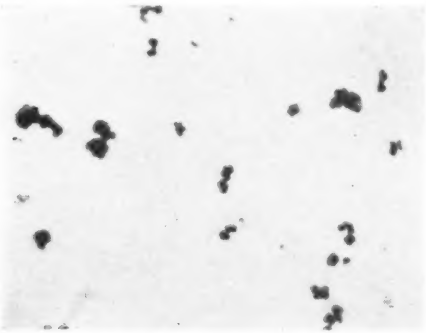

Fig. 4
Multicoccoid form of organisms, having from 4 to 20 coccoid bodies within a cytoplasm-like structure.

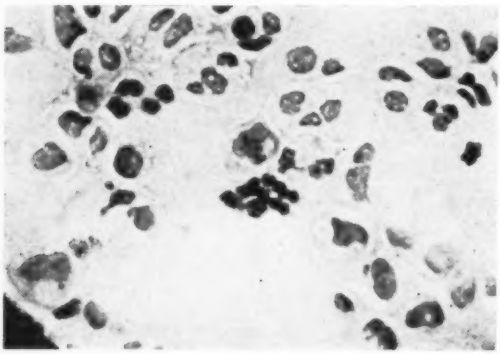

Fig. 9
Smear from growth on chorio-allantoic membrane of chick embryo.

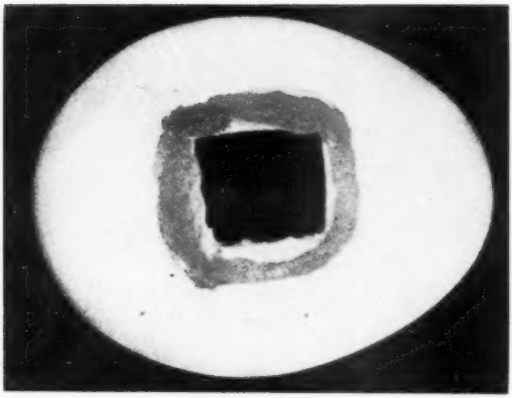

Fig. 5
Embryo containing egg with a small window for inoculation, rimmed with vaseline-paraffin.

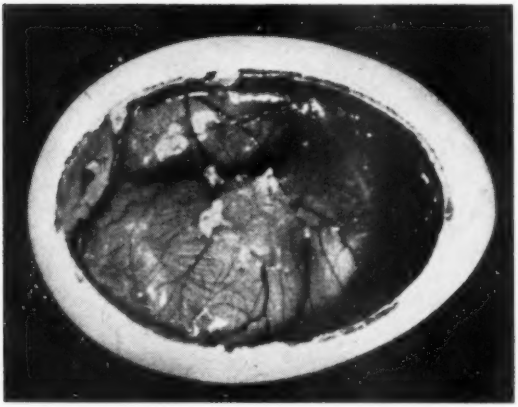

Fig. 6
Shell removed from inoculated egg showing chorio-allantoic membrane. In the center the thickened area is the growth of the organism.

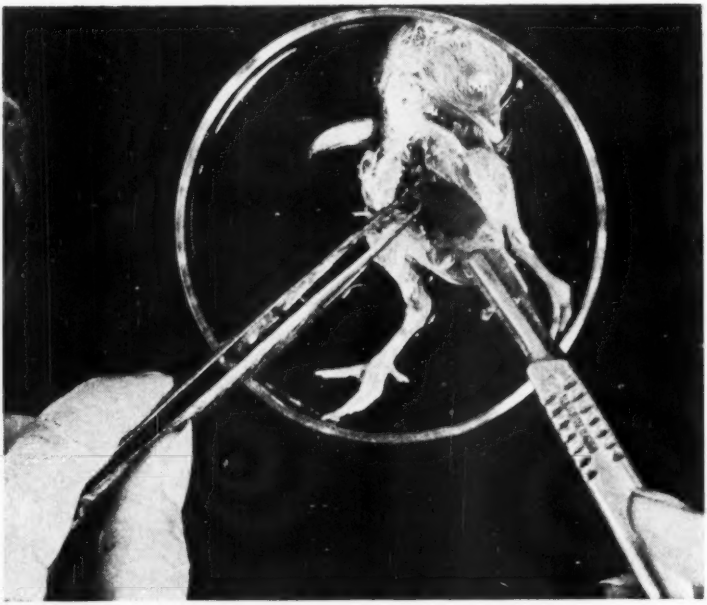

Fig. 7
Heart's surface is sterilized by searing it with a red-hot knife blade.

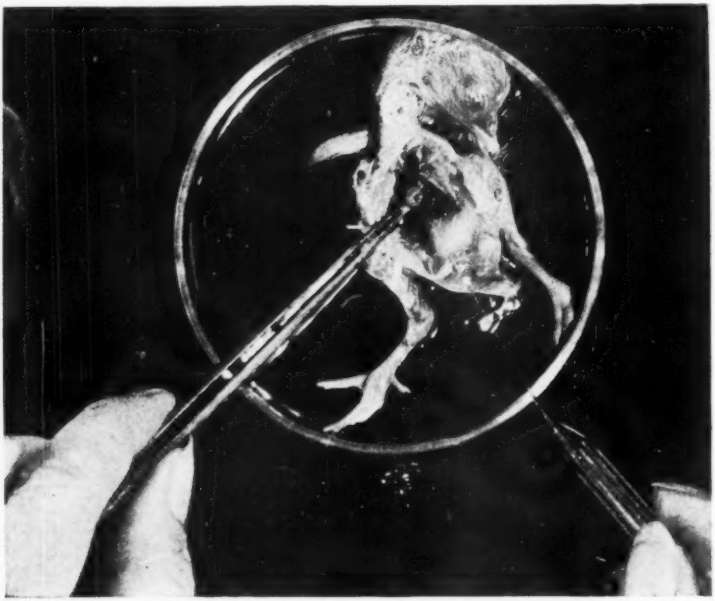

Fig. 8
This seared area is pierced through with a capillary pipette.

apparent on survey of the results as a whole. However, the 5th day sacrifice was made from the C generation, the 6th day sacrifice from the G generation and the 7th day sacrifice from the F generation.

Discussion

Dodge (7) describes the cryptococcus as cells spherical, ovoid, or ellipsoid, occurring singly or held in more or less irregular groups by the secretion of thick gelified capsules, specially in old age. Not forming ascospores, mycelium or pseudomycelium. Liquifaction of gelatin very slow when present. No fermentation and acidity rare with carbohydrates. On liquid media, pellicle thick, formed by coalescence of slimy, floating islets when present, sediment usually slimy. He also states that cryptococcus should be used for a residue species after other valid genera have been removed. Our organism seems to fit the foregoing description; no gas formed by fermentation on sucrose, lactose, maltose, mannose, glucose and fructose but acid was formed in each case, no pellicle formed in any liquid media but a slimy sediment in broth and in sucrose, lactose and maltose broth. No ascospores, mycelium or psueedomycelium have been present to date.

Dodge states, "Up to the present only about half the pathologists who reported cases have described cultures of the organisms isolated, and not in a single case has it been adequately described, while the only adequate description is not accompanied by case histories or pathology." We have attempted to faithfully describe what we have done and seen in carrying out the technical procedures for the identification of this organism unknown to us and to our pathology staff.

The cultivation of the organism in living tissue of the chorioallantoic membrane of the chick embryo has produced an exudate showing a similar picture to that seen in the sputum of the patient (Compare Fig. 1 and Fig. 9). The intracellular organisms are usually the multicoccoid form in both cases. In the living media I found that sometimes the multicoccoid form reverted to the uninuclear form without an intervening transfer; on inert media this never occurred except after transfers.

The specific and general results in the use of the chick embryo were interesting and gratifying and the author wishes to express her

appreciation for the assistance and criticisms of the pathologists, Dr. Carl W. Maynard and Dr. Mae Gallavan, in the initiation and carrying out the entire technical procedures, together with the recording of results, for the purpose of the identification of a yeast-like organism.

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THE WHITE BLOOD CELL PICTURE IN VIRUS DISEASES WITH SPECIAL REFERENCE TO COLORADO TICK FEVER*

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In recent years more attention has been given to diseases caused by viruses particularly since the advent of methods which make it possible to study the infectious agent. Perhaps the diagnosis of these conditions can be made without the help of the laboratory but in the course of routine blood counts the medical technologist will surely encounter these diseases and will wonder what the typical blood cell findings will be. Little if any change is noted in the erythrocyte or hemoglobin values but various deviations from normal are found in the numbers of leucocytes. Considerable work has undoubtedly been done but in perusing the standard laboratory text books (1, 2, 3, 4, 5) not a great deal is found concerning the white blood cells affected in the virus diseases of which there are some 15 or 20. Pepper and Farley (3) in their Practical Hematological Diagnosis state that the diseases caused by neurotropic virus may at times show a moderate neutrophilic leucocytosis while those with a virus of ectodermal cytotropism tend to have an early transitory leucocytosis followed by a definite fall to normal or below. This trend is shown in the following consideration of some of the better known diseases of virus origin.

Probably the most common of the virus infections is influenza. Most authorities agree that leucopenia is the rule, sometimes with an increase in lymphocytes and a decrease or total absence of eosinophils. Complications may cause a leucocytosis but leucopenia often persists even in bronchopneumonia. Virus pneumonia at first shows a leucopenia shifting to a leucocytosis.

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* *Presented at the Annual Meeting of the American Society of Medical Technologists, July* 11, 1944, *Chicago, Ill.*

Among the common communicable diseases there is measles with a leucopenia and a high proportion of monocytes during the height of illness and a lymphocytosis during convalescence; mumps and chicken pox, a leucocytosis with a relative increase in lymphocytes. Smallpox has a slight transitory neutrophilic leucocytosis but varies depending on the stage of the disease and usually there is a leucopenia toward the end.

Poliomyelitis shows a moderate leucocytosis with a relative neutrophil increase as do herpes zoster and rabies. In psittacosis there is a normal white blood cell count at the beginning of illness, then a transient leucocytosis which is followed by leucopenia. Encephalitis lethargica and acute choriomeningitis have a leucocytosis with an increase in neutrophils; and lymphogranuloma inguinale a leucocytosis with a shift to the left.

With the advent of the present conflict more attention is being given to the so-called tropical diseases. Among these insect borne conditions which we may encounter there are several caused by a virus. Leucopenia is typical of phlebotomus or sandfly fever. There are different reports on yellow fever, some authorities state that a leucocytosis is the rule while others report a leucopenia.

One of the most complete hematological studies in the literature is reported by Simons, St. John and Reynolds (6) on their study of dengue fever, a mosquito borne virus disease. They carried out daily studies on the leucocyte picture and report a characteristic leucopenia with a shift to the left of the granulocytes. Kisner and Lisansky (7) recently confirmed these findings in a study of an epidemic of dengue fever among army personnel on a South Pacific island. In two of their cases with intercurrent infections there was a conversion of the leucopenia to a leucocytosis. As well as the shift to the left they reported finding abnormal lymphocytes with a vacuolated cytoplasm and coarse granular inclusions.

Rift Valley fever, another virus disease transmitted by the mosquito, also shows a leucopenia, the polymorphonuclear neutrophils being affected. The leucopenia persists into convalescence.

A comparatively recently described clinical entity in the Rocky Mountain region is Colorado tick fever considered a tick borne disease probably caused by the bite of Dermacentor andersoni. The

etiology is, as yet, unknown but work done suggests it may be caused by a virus. It was found by Topping, Cullyford and Davis (8) that patients suffering from this condition showed a consistently lowered white blood cell count without any decided shift in cell distribution. During the course of experimental work on this condition, we followed 14 volunteers who had the disease after having been inoculated with infective serum. The volunteers were either medical students or medical school personnel and were known to have normal white blood cell pictures. At the time the experiment was begun, a study of the white blood cells revealed normal values in every case. With the beginning of symptoms a daily study of the leucocytes was made, the specimens being taken at approximately the same time of the day. The white blood cell counts were done in duplicate using standardized equipment. In each volunteer with the beginning of symptoms the total white blood cell count began to drop and reached a low point of approximately 2,000 cells per cu. mm. at the beginning of the second attack. The lowest count was 1,200 leucocytes per cu. mm. Then the total leucocyte count slowly increased but did not return to normal for 5 to 7 days after clinical recovery. Using humans as experimental subjects we wondered what would happen should a secondary infection occur. One volunteer developed an upper respiratory infection and his leucocyte count immediately increased.

Blood smears made at the same time the leucocyte count was taken were stained with Wright's stain and a Schilling hemogram made on at least 300 cells. To show what type of cells were affected by this decrease in total number, the counts were reported in absolute numbers as well as in per cent.

Although there were individual variations in the relative values of the polymorphonuclear neutrophils, every case showed an absolute decrease in the total number of these cells. This picture continued during the course of the disease and until the total white blood cell count returned to normal. There was a definite shift to the left. At the time the total white blood cell count was the lowest, in many of the cases the band forms outnumbered the segmenters. Occasionally a metamyelocyte was present but younger cells in the series were never seen. Toxic granules were rarely observed.

The lymphocytes showed similar variations in the relative values and likewise an absolute decrease in the total number. They appeared as normal small cells during the disease but during convalescence large, immature cells were seen resembling the atypical lymphocytes of infectious mononucleosis. At this same time, but appearing in the cytoplasm of normal large lymphocytes we observed bodies which we have never seen before in peripheral blood smears. These basophilic staining bodies resembled the nucleus of the cells in which they appeared although the nucleus showed no evidence of disintegration. They varied in size and measured from 0.4 to 1.6 micra in diameter and were usually round although a few were slightly oval. From 2 to 8 bodies appeared in the cells and they were often in pairs. The nature of these bodies has not been determined.

There was a slight increase in the per cent of monocytes. In actual numbers they were normal or only slightly increased. Eosinophils decreased in numbers or dropped out during the febrile attacks and no change was observed in the basophils.

As the total white blood cell count returned to normal the lymphocytes tended to increase more rapidly than the granulocytes. It will be seen that in Colorado tick fever there is a characteristic leucopenia throughout the illness and all types of leucocytes are decreased in total numbers.

Kracke (2) states that the mechanism by which leucopenic states are produced is not understood but is usually attributed to the action of bacterial toxins on the bone marrow. Colorado tick fever is a comparatively mild condition and the blood picture seemed out of keeping with the severity of the disease. The question naturally arose what effect the infective agent might have on the bone marrow. On two of our volunteers, sternal marrow was obtained at the time their white blood cell count was lowest. The preparations were studied in Giemsa stained sections; Wright's stained smears and with supra vital stained preparations. Counts of 500 cells were made on each of the fixed stained preparations. The proportions and appearance of the myeloid elements were normal. No conclusions can be drawn from this study of only 2 cases but it seems there is no deviation from normal in the bone marrow picture.

Although daily studies were not carried out on all of the cases of the naturally acquired disease, the counts that were performed showed an identical picture to that observed in our experimental series.

Besides establishing the typical white blood cell findings in Colorado tick fever by daily counts, this study has again called to mind several interesting phases in "blood counting." The admission or routine blood count may not reveal the typical picture of the disease concerned but on the other hand may definitely assist the physician in making a diagnosis.

More consideration should be given to the distribution of the leucocytes and a Schilling hemogram is well worth the extra few minutes it may take.

The present system of reporting the differential count in per centile values does not always depict the true situation. More and more the medical technologist will be called upon to report the differential count in absolute numbers and in this way the physician is able to see whether the leucocytosis or leucopenia is due to an increase or decrease in any particular type of cell.

Although it is not always feasible to carry out daily blood studies, our work on Colorado tick fever has shown the importance of a hemogram and often was invaluable in making the diagnosis.

Summary

The white blood cell picture in a number of virus diseases is reviewed from the literature. Diseases caused by neurotropic virus usually show a moderate neutrophilic leucocytosis and those with a virus of ectodermal cytotropism tend to have an early transitory leucocytosis followed by a definite fall to normal or below.

The white blood cell findings on 14 cases of experimental Colorado tick fever are presented. Daily white blood cell and differential counts were done on each case during the illness and through convalescence. The typical picture was a definite decrease in the total number of leucocytes and all types of cells were affected. The counts did not return to normal for about a week after clinical recovery.

There was a definite shift to the left of the granulocytes most pronounced when the total white blood cell count was the lowest. Large, basophilic staining bodies appeared in the cytoplasm of normal appearing lymphocytes during convalescence.

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LABORATORY PROCEDURES FOR DEMONSTRATING TUBERCLE BACILLI USED AT GLEN LAKE SANATORIUM, OAK TERRACE, MINNESOTA*

By CATHERINE HANITCH, B.S., M.T. (A.S.C.P.)

Preparation of Smears

Mucopurulent particles, if present, are picked up with the broken ends of applicators of wood. The material is spread thinly and evenly over two-thirds of a slide. The spreading is done with the side of an applicator, heating the slide gently once or twice while doing this, care being taken not to overheat the slide or the material will flake off when rubbed. The surplus material is rubbed off on an applicator.

In making smears from body fluids, the material is centrifuged and any excess liquid is absorbed with a cotton swab. The sediment is shaken down onto the slide or smeared on with the end of a broken applicator. The material is rubbed constantly until it has dried on the slide. This may be hastened by gently heating the slide during the process, which helps in preventing the material from peeling off during the staining.

Enough absolute alcohol is added to spinal fluids with a low cell count to give the liquid a cloudy appearance. The tubercle bacilli will be carried down with the coagulated material when centrifuged.

New slides only are used for smears in order to avoid the restaining of bacteria remaining on the glass from a previous smear. It also eliminates numerous scratches which may catch the red stain and give the appearance of an acid-fast bacillus, but on more careful examination, can be readily distinguished from acid-fast organisms.

Presented at the Annual Meeting of the American Society of Medical Technologists, June 11, 1944, Chicago, Ill.

Staining of Smears

F. B. Cooper's[1] Ziehl-Neelsen Modification

Carbol Fuchsin

Saturated alcoholic solution of basic fuchsin (approx. 3 gms. pure crystals, Coleman & Bell, in 100 c.c. of 95% alcohol.)	10 c.c.
Aqueous solution of phenol (5%)	90 c.c.
Filter	
Aqueous solution of sodium chloride (10%)	3 c.c.

Brilliant Green Counterstain

Brilliant green	1 gm.
1/10,000 aqueous solution of sodium hydroxide	100 c.c.

Acid Alcohol

Ethyl alcohol	95 c.c.
Nitric acid (conc.)	5 c.c.

The slides are steamed four minutes with carbol fuchsin or left over night in the stain at 37° C. These are cooled to room or ice-box temperature. (This step is important.) The slides are washed with tap water and destained with acid alcohol. Then they are washed with tap water. Next they are counterstained with brilliant green for one minute. Frequently the organisms can be located with high dry power and then examined with oil immersion lens.

The addition of the saline solution tends to precipitate more dye with each tubercle bacillus, thus making it more readily seen. The nitric acid must be used in the alcohol rather than hydrochloric or the effect of the saline is lost. The green counterstain makes an excellent background, since the numerous secondary organisms do not stand out as definitely as they do with methylene blue.

The dye has a tendency to precipitate in a month or two after the saline has been added to the stock solution of carbol fuchsin. A Copelin jar may be filled to the top of the grooves with the stock solution and 10% saline is added with a medicine dropper marked for delivering 1 c.c. The solution is stirred with an applicator and set aside for two days to allow a filterable precipitate to settle out before using.

The stained smears are blotted with fresh sheets of filter paper to prevent any bacteria from being carried over from one slide to another.

Sputum Examination for Tubercle Bacilli

Approximately 5 c.c. of sputum is poured into a round bottom, 50 c.c. centrifuge tube. If the sputum is too thick, or an insufficient quantity, it is diluted and made up to 5 c.c. with distilled water. It is then plugged with non-absorbent cotton and set in an incubator at 37° C. for from 48 to 72 hours.[2, 3]

After incubation, to the sputum is added an equal amount of 4% sodium hydroxide (45 gms. sodium hydroxide and 2 gms. potassium or ammonia alum in 1000 c.c. of distilled water to which has been added enough phenol red powder on the end of an applicator to give it a definite pink color. This is agitated in a shaking machine for ten minutes or mixed thoroughly with about a half dozen applicators. It is neutralized with 4% hydrochloric acid (111 c.c. conc. acid in 899 c.c. of distilled water). It is made up to approximately 40 c.c. of distilled water and centrifuged at high speed for ten minutes. The supernatant fluid is poured off and the sediment is smeared on a slide.[4]

Direct smears made from mucopurulent material may be negative while the smears made from the concentrated sediment of the same specimen may have almost numerous tubercle bacilli. Sputums, which have become digested or have an infiltration of mucopurulent material in the mucus, may be expected to yield more positive results when concentrated. If the sputum is scant in quantity with only a few specks of mucopurulent material, a direct smear is the most satisfactory method of examination, since there is little or no precipitate left after concentration. There is a lack also of precipitate from saliva or a small amount of saliva and mucus.

Preparation of Material for Guinea Pig Inoculation

Approximately 5 c.c. of sputum (diluted with distilled water if insufficient liquid or thick material) is poured into a round bottom centrifuge tube and placed in an incubator at 37° C. until the specimen has reached the same temperature. An equal amount of 4% sodium hydroxide is added and then the fluid mixed thoroughly with several applicators, returned to the incubator for forty minutes,

stirring twice during that period. The liquid is then neutralized with 4% hydrochloric acid. After making it up to approximately 40 c.c. with distilled water, it is centrifuged for ten minutes at a high rate of speed. The supernatant fluid is poured off and 40 c.c. of distilled water is added. It is mixed, centrifuged again, and the supernatant fluid poured off. Several cubic centimeters of distilled water are added, remixing thoroughly with applicators so that the precipitate will be fine enough to pass through the needle of the syringe. The specimen is poured into a sterile 5 c.c. syringe with a needle (one inch, 21 gauge) attached, with the point of the needle covered with a wad of cotton.

The same method is used for a stool specimen with the exception that the neutralized material is filtered through five thicknesses of gauze into another centrifuge tube, before centrifuging.

A urine specimen is prepared the same way as a sputum, but it is centrifuged after incubating. When the supernatant fluid is poured off, a few cubic centimeters of distilled water are added with enough phenol red on the end of an applicator to color the liquid pink. Then it is mixed and neutralized. The liquid is made up to approximately 40 c.c. with distilled water and centrifuged again. Then the procedures described above are repeated.

All other specimens are injected untreated with the exception of those containing virulent organisms other than tubercle bacilli. Such specimens are treated like the sputum. Thick pus is diluted with normal saline. Specimens with an excessive amount of blood are laked out with distilled water and centrifuged. Tissues are ground in a sterile mortar with a small amount of sterile sand and later diluted with normal saline. The sand is allowed to settle out and the supernatant fluid is sucked off through a needle into a syringe ready for inoculation.

When performing intraperitoneal inoculations on guinea pigs, the animal is held firmly enough to avoid excessive movement, and to keep the skin of the abdomen taut. The needle is pushed about one-half inch into the abdominal cavity at a slant. Novices may place the forefinger about half-way down the needle to help judge the distance it may be inserted to prevent the needle from entering too far.

Guinea pigs are inoculated intraperitoneally and autopsied six weeks after the last injection. A single pig is frequently given as many as four inoculations, spaced one week apart, using different specimens of material from the same source.

This laboratory has been slightly more successful with positive findings on urine specimens injected into guinea pigs than in inoculations made on culture media.

Stool specimens are more successfully injected into pigs than cultured because of the large number of secondary organisms, yeasts, and molds, that they contain.

Vaginal and ear washings may have non-pathogenic acid-fast bacilli which may be ruled out by injecting them into pigs.[5]

Cultural methods are also used for the demonstration of tubercle bacilli but I have not taken up this method in my paper.

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ABSTRACTS

THE EFFECTIVENESS OF DIFFERENT CULTURE MEDIA IN THE ISOLATION OF ENTERIC MICRO-ORGANISMS: E. R. Neter and P. Clark, Am. Jr. Dig. Dis., vol. 11, No. 7, July '44, pp. 229.

Of 500 consecutive stool specimens, 324 contained organisms other than B. coli, B. aerogenes and intermediates. The following organisms were also found: B. typhosus, B. paradysenteriae (Flexner), B. schmitzii, B. castelanii, B. paratyphi B, B. typhi murium, B. thompson, B. proteus, B. morganii type I, paracolon bacilli, B. pyocyaneus, B. alcaligenes & B. coli anaerogenes. In 73 instances these organisms were isolated from only one out of 5 culture media. This bears out the suggestion that it is necessary to use several differential media simultaneously for best results. The culture media used were: Shigella-Salmonella agar, desoxycholate citrate agar, enriching fluid and Endo and MacConkey agar.

THE THERAPEUTIC USE OF THE AMINO ACID HISTIDINE IN ALLERGY AND SHOCK. HISTIDINE AS A FACTOR IN HISTAMINE-EPINEPHRINE BALANCE: S. L. Ruskin, Am. Jr. Diges. Dis., vol. 11, No. 7, July '44, pp. 209.

Since histidine is antagonistic to histamine, it is important in histamine-adrenalin balance in shock and may also be a useful therapeutic agent in allergy and related conditions. In post-operative treatment it would be valuable for the feeling of well being and energy that it imparts. Further work on the addition of histidine to parenterally administered protein hydrolysates as blood substitutes has been begun.

GREENISH PIGMENTATION OF NAIL PLATES FROM BACILLUS PYOCYANEOUS INFECTION: Report of Two Cases. L. Goldman and H. Fox, Arch. Derm. and Syph., vol. 49, No. 2, Feb. '44, pp. 136.

One of these cases began with a burn and the other with a contact dermatitis. Infection with B. pyocyaneous was secondary in both instances. They are unusual in that the soluble pigments of a saprophyte colored the nail plates a bright green. The color later changed to a dull green. In one case it persisted for 6 months.

BOOK REVIEW

TABER'S DICTIONARY OF GYNECOLOGY AND OBSTETRICS: By Clarence Wilbur Taber, Medical Editor and Author of Cyclopedic Medical Dictionary, Condensed Medical Dictionary, etc., and Mario A. Castallo, M.D., F.A.C.S., Assistant Professor of Obstetrics, Jefferson Medical College; Gynecologist to St. Mary's and St. Agnes' Hospitals; Obstetrician to St. Mary's Hospital; Diplomate, American Board of Obstetrics and Gynecology, etc., etc. F. A. Davis Company, Philadelphia, 1944.

Taber and Castallo have designed a specialized dictionary primarily for the use of the gynecologist, obstetrician and obstetrical supervisor. This glossary has a far wider appeal than the specialty it covers since innumerable terms relating to instruments, apparatus, procedures and appliances are recorded between "Abderbaldens reaction" at the beginning and the last word "zygotoblast."

A "fact-finding index" features the opening section in which key words are listed, such as "abdomen" with a collateral: "changes in pregnancy," to show by designated markings whether the collaterals appear in the alphabetic section as principals or are to be looked for under the key word. This is a helpful guide when, for example, a search is being made for information on "metabolism in pregnancy" the special index shows that this feature of pregnancy is listed under "metabolism" rather than under "pregnancy."

The Taber and Castallo lexicon not only should serve well the express purpose but also offers a comprehensive medical dictionary and source book of information for everyone associated with medical activities.

STATE AND LOCAL SOCIETIES

Philadelphia in June

Soon we will be meeting in Philadelphia for another convention. Gather up all that data you have been collecting and write that paper! If you will have a paper to present or any suggestions, let us know. Mabel O. Stewart, Chairman, Program Committee, 4200 East 9th Avenue, Denver 7, Colorado.

NOTICE

From the office of the Executive Secretary

Since July 1st, 1944, 200 technologists have joined the American Society of Medical Technologists. This swells our total membership to 1,000, as of January 1, 1945.

We note that some states have a very small percentage of Technologists belonging to the National Organization (see Roster). May we ask that you call attention to this fact among Medical Technologists of your own state.

Our National Convention will be held in June of 1945. More exact information concerning the date and locality will be given in the March *Journal*. The sum of $200 will be given as prizes along with the gold, silver, and bronze medals for the three best scientific papers and the most outstanding scientific exhibit.

Many of the *Journals* sent out are being returned. This is the result of the failure of members in notifying this office when changing localities. May we ask that you be more prompt to inform us of your change of address.

Colorado

For the December meeting of the Colorado Society of Medical Technologists a Christmas party was held at the Childrens' Hospital with 45 technologists attending.

In January we will be serious again and begin a series of lectures and laboratory work in parasitology. The medical technologists in

Pueblo, Colorado,, have organized as a branch of the Colorado Society and are holding regular monthly meetings with Dr. C. W. Maynard as their sponsor, Pauline Kurachi, president, and Lavina White, secretary.

Minnesota

Minnesota has about 160 registered medical technologists employed in the state and of these only seven have never taken membership in the state organization. Through the instrumentality of Miss Frieda Claussen this splendid piece of work has been accomplished.

While Minnesota is not districted for medical technologists it happens that the northern part of the state keeps its interest up through the activities of a local organization known as The Arrowhead Society of Medical Technologists. The second active organization is that in and about St. Paul known as The Twin Cities' Society of Medical Technologists. Both local societies are trying to increase national membership and up-to-date there has been a commendable response. In an all-out attempt to make the state conscious of the American Society of Medical Technologists Miss Frieda Claussen will address the medical technologists of the northern part of the state in Duluth in March, 1945.

The Administrative Board of the Baruch Committee on Physical Medicine has announced the granting of an additional total sum of $185,000, which is being given by Mr. Bernard M. Baruch for the further advancement of the program in physical medicine and the physical rehabilitation of those disabled in the war. This sum has been divided into seven grants as follows: $50,000 to the Massachusetts Institute of Technology, Cambridge, Massachusetts; $40,000 to the Medical School of the University of Minnesota, Minneapolis, Minnesota; $30,000 to the Medical School of Harvard University, Boston, Massachusetts; $30,000 to the Medical School of the University of Southern California, Los Angeles, California; $15,000 to the Medical School of the University of Iowa, Iowa City, Iowa; $15,000 to the Medical School of the University of Illinois, Chicago,

Illinois; $5,000 to Marquette University Medical School, Milwaukee, Wisconsin.

The grants to Massachusetts Institute of Technology and the University of Minnesota are in addition to the gift of $1,100,000 made by Mr. Baruch in April of 1944, at which time grants were made to Columbia University College of Physicians and Surgeons, New York University College of Medicine, the Medical College of Virginia and for minor research and fellowship programs for the advancement of physical medicine.

The present gift to Massachusetts Institute of Technology is in support of a five-year program of training and research in electronics, instrumentation and physics in relation to medicine, to be carried on under the auspices of the Department of Biology and Biological Engineering. It was the conviction of the Scientific Advisory Committee of the Baruch Committee on Physical Medicine that Baruch Fellows and other physicians should have more than a superficial knowledge of the physics and technology underlying the physical methods and instrumentation used in this field and it was suggested that training in this aspect might effectively be centered at the Massachusetts Institute of Technology. The program will be under the general supervision of Doctor Francis O. Schmitt, head of the department of biology and biological engineering and under immediate supervision of Doctor K. S. Lion, assistant professor of applied biophysics, who is an expert in physical instrumentation.

The grant of $40,000 to the University of Minnesota is to support the development of a three-year teaching and fellowship program in physical medicine. The primary objective of the program is to be the furtherance of fundamental training of research workers and teachers. The program has as its basis the development of scientists in the field of physical medicine. As an auxiliary to this basic training will be developed facilities for the training of clinicians and technicians.

The other grants have been allocated from the fund of $200,000 given by Mr. Baruch in April. The sum of $30,000 was granted to Harvard University Medical School for establishment of a three-year program to provide fellowship or residencies to be used for the benefit of qualified physicians who are selected to be trained in

this field. This sum will be administered by a strong standing committee on physical medicine recently appointed by Dean C. Sidney Burwell of the Harvard Medical School, composed of Doctor J. B. Ayer, Doctor D. Denny-Brown, Doctor W. T. Green, Doctor J. H. Means, Doctor A. L. Watkins and Doctor E. M. Landis (Chairman). Appointments to the fellowships, which generally carry stipends of $2500, will be made annually but may be renewed to provide up to three years of specialized study and research. Emphasis will be placed upon training a few men in basic research and clinical investigation.

Unusual opportunities for clinical experience and research in the psychologic and psychiatric aspects of physical medicine will be available at Harvard. The first year will be wholly or in part devoted to basic research related to physical medicine in one of the pre-clinical sciences such as physiology, anatomy or biophysics. The second year will be spent in clinical training in physical medicine at the Massachusetts General Hospital and other hospitals affiliated with the Harvard Medical School. In the third year, fellows will be assistants in physical medicine with clinical responsibilities. For candidates with extensive previous training, one-year clinical fellowships will also be granted. Applicants must have an M.D. degree from an approved medical school and a minimum of one year of internship in an approved hospital. Applications may be obtained from the Dean, Harvard Medical School, 25 Shattuck Street, Boston 15, Massachusetts.

The sum of $30,000 is granted to the University of Southern California to inaugurate a program of teaching and research in physical medicine in its medical school. The sum of $15,000 is granted to the University of Illinois to inaugurate a teaching program in physical medicine at its medical school. The sum of $15,000 is granted to the Medical School of the University of Iowa to assist in a joint research and teaching program concerning the effectiveness of different methods of applying heat to the deep tissues of the human body. Finally, the sum of $5,000 is granted to the Medical School of Marquette University, Milwaukee, Wisconsin, for continuance of research in the physiology and pathology of nerves and muscles as related to physical medicine.

In discussing these grants Doctor Frank H. Krusen, the director of the Baruch Committee pointed out that Mr. Baruch had been particularly interested in the important field of electronics as applied to medicine and he said that the center at Massachusetts Institute of Technology gave promise of revolutionizing the application of electronics in the diagnosis and treatment of the sick. Doctor Krusen expressed gratitude concerning the establishment of fellowships in physical medicine at Harvard and mentioned the advantage to the field of physical medicine in having this great center assume leadership in the training of fellows. He also stated that the aid given to the University of Minnesota and the University of Southern California would extend the activities of the Baruch Committee into the midwest and far west and thus tend to strengthen this important program. In conclusion, Doctor Krusen announced that the Administrative Board does not contemplate the recommendation of any further large grants for the establishment of additional departments of physical medicine in our medical schools. He said that the Baruch Committee would now turn its main attention toward the adequate development of the centers already established, toward providing advice in the organization of proper teaching of physical medicine in medical schools and, through its strong Committee on War and Postwar Physical Rehabilitation and Reconditioning would attempt to promote proper development of physical medicine in the rehabilitation and reconditioning of both military and civilian casualties of war. The Board agreed that Mr. Baruch's gifts had served as a means of providing prompt coordination of the entire program for rehabilitation of our wounded and for the provision of the trained personnel so greatly needed in activating this program.

The 1944 epidemic of infantile paralysis has officially become the second worst in the recorded history of the disease in the United States, it was announced today by Basil O'Connor, president of The National Foundation for Infantile Paralysis.

At the same time, Mr. O'Connor stressed the need for more skilled polio fighters, especially physical therapists, and urged that men and women who have the proper qualifications make applica-

tions for scholarships offered by the National Foundation and its Chapters.

In the first 41 weeks of 1944, or up until October 14, there were 16,133 cases of poliomyelitis, according to the latest report from the U. S. Public Health Service. This is 353 cases more than were reported in the country for 1931 which previously had been the second worst year for the disease. The all-time record was in 1916 when there were 27,621 cases.

"Although the peak of the outbreak was passed more than a month ago, the epidemic itself has not yet ended," warned Mr. O'Connor. He pointed out that there were 710 new cases reported for the week of October 7-14, or nearly half the weekly total at the peak of epidemic, the week ending September 2 when 1,683 cases were reported.

"This great outbreak has tested not only the resources of the National Foundation and its Chapters, but also those of the nation," he added. "The National Foundation's greatest problems were in obtaining sufficient doctors, physical therapists and professional personnel to cope with nearly simultaneous outbreaks in widely separated sections of the south, the east and the middle west. Seven skilled polio doctors, 65 physical therapists and nearly 10 tons of wool for use in hot pack treatments were rushed to stricken areas by the National Foundation. All 26 respirators owned by the National Foundation are still in use in epidemic areas. At the request of the National Foundation, the American Red Cross recruited more than 700 nurses from all parts of the country to staff regular and emergency hospitals."

The seven states most severely menaced were New York, North Carolina, Pennsylvania, New Jersey, Virginia, Ohio and Kentucky, but emergency aid in the form of money, professional personnel and supplies has been sent this year by the National Foundation to 21 states and the District of Columbia.

"Although the National Foundation and its Chapters have trained many physical therapists in the modern principles of treating infantile paralysis, many more technicians are still needed for this present fight," said Mr. O'Connor. "The greatest handicap in rendering effective aid in any epidemic of infantile paralysis has been

the lack of physical therapists. The National Foundation for Infantile Paralysis through its scholarships in accredited schools of physical therapy has been and still is seeking to enlarge this first line of defense.

"These scholarships sponsored by the National Foundation are available to graduate nurses, graduates in physical education or those with a minimum of two years undergraduate college work with science courses. Such applications may be made through the National Foundation or to The American Physiotherapy Association, 1790 Broadway, New York 19, N. Y.

"The field of physical medicine is expanding rapidly and this is an opportunity for men and women to enter an interesting lucrative profession with a chance of performing a humane service."

Name of State or Local Society	A.S.M.T. Affiliation	Meetings	Secretary's address
Caddo Parish Society of M. T. 1944-'45 Pres.—Eola Kendrick	non-aff.	monthly	Harriet Cypert, M.T. 179½ Fremont Street Shreveport 67, La.
California Association of M. L. T. (Santa Barbara Chapter) 1943-'44 Pres.—Grace P. Butera	non-aff.	monthly	Florence Connelly, M.T. 317 West Pueblo Str. Santa Barbara, Calif.
Chicago Society of M. T. 1944-'45 Pres.—Dorothy Laestar	aff.	monthly	Ada Meloy, M.T. 1575 Florence Ave. Evanston, Ill.
Colorado Society of M. T. 1944-'45 Pres.—Mabel O. Stewart	aff.	monthly	Annalee Breslford, M.T. 1560 High Street Denver, Colorado
District of Columbia Society of M. T. 1943-'44 Pres.—Zanerian E. Funk	aff.	monthly	Evelyn F. Ballou, M.T. 4105 Third Street N. W. Washington, D. C.
Illinois Society of Clinical L. T. 1944-'45 Pres.—Fannie Warnock	aff.	semi-ann.	Edna H. Murmann, M.T. 3924 N. Monticello Ave. Chicago, Ill.
Indiana State Society of M. T. 1944-'45 Pres.—Virginia Sue Alley	aff.	annual	Hazel M. Childs, M.T. Indianapolis City Hosp. Indianapolis, Ind.
Indianapolis Society of M. T 1944-'45 Pres.—Nila Maze	aff.	monthly	Ruth Trotter, M.T. 615 East Dr., Woodford Pl Indianapolis, Ind.
Inland Empire Society of M. T. 1943-'44 Pres.—Frances Premo	non-aff.	annual	Lenore De Vor East 547 Gordon Spokane, Wash.
Little Rock Society of M. T. 1943-'44 Pres.—Marcella Drilling	non-aff.	quart.	Lila Church 2116 Orange Street Little Rock, Ark.
Minnesota Society of M. T. 1944-'45 Pres.—Mr. Chauncey Winbigler	aff.	annual	Thelma Erickson, M.T. 1211 East 4th Street St. Paul 6, Minn.
Nebraska Society of M. T. 1943-'44 Pres.—Romona Forbes	aff.	annual	Marjorie Lundeen, M.T. Lincoln General Hospital Lincoln, Nebraska
New Hampshire Society of M. T. 1943-'44 Pres.—Sister Marie-Rose (Larivee)	non-aff.	annual	Marion P. MacMartin, M.T. Mary Hitchcock Mem. Hosp. Hanover, N. H.

Name of State or Local Society	A.S.M.T. Affiliation	Meetings	Secretary's address
Niagara Frontier Association of M. T. 1943-'44 Pres.—Wilma Riehle	aff.	monthly	Mrs. Angela Auer, M.T. 16 Duerstein Ave. Buffalo, N. Y.
Ohio Society of M. T. (Akron) 1943-'44 Pres.—Kathryn Teeple	aff.	annual	Mary Benedict Clark, M.T. Nichols General Hosp. Louisville, Ky.
Ohio Society of Clinical L. T. Dist. #1 1944 Pres.—Jean Jones	non-aff.	semi-ann.	Patricia Nelan, M.T. c/o City Hospital Akron, Ohio
Oklahoma Society of M. T. 1944 Pres.—Marie Clark	aff.	semi-ann.	Lelia Woodworth, M.T. 604 S. Cincinnati Tulsa 3, Okla.
Omaha-Council Bluffs Society of M. T. 1943-'44 Pres.—Kathern Belle Forest	non-aff.	bi-mo.	Josephine Benal, M.T. City Health Dept. City Hall, Omaha, Nebr.
Pennsylvania Society of M. T. 1944-'45 Pres.—Ellen Marie McDevitt	aff.	monthly	Anne Caverly, M.T. 5000 Pulaski Avenue Philadelphia 44, Penna.
Savannah Society of M. T. 1943-'44 Pres.—Sadie Cartwright	aff.	monthly	Jurelle S. Hooper, M.T. 20 East 56th Street Savannah, Ga.
Tulsa Round Table of M. T. 1944 Pres.—Oscar Stewart	non-aff.	bi-mo.	Lythene Vermillion, M.T. Hillcrest Memorial Hosp. Tulsa, Okla.
Wisconsin Association of M. T. 1943-'44 Pres.—Alice A. Thorngate	aff.	semi-ann.	Mrs. Elizabeth Kullman, M 2460 South 59th Street Milwaukee 14, Wisc.
Wisconsin Association of M. T. Milwaukee District, 1943-'44 Pres.—Dorothy Zoeller	non-aff.	monthly	Esther Lemont, M.T. 2618 North Summit Ave. Milwaukee 11, Wisc.

(aff.—affiliated with A. S. M. T.)
(non-aff.—not affiliated with A. S. M. T.)
Key: (semi-ann.—semi-annual)
(bi-mo.—bi-monthly)
(quart.—quarterly meetings)

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American Journal of Medical Technology

ROSTER

American Society of Medical Technologists

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Herring, Flora Mitchell, 930 South 20th St., Birmingham.
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